I. INTENDED USE

The Diagnostic Hybrids, Inc. D³® Ultra 8™ DFA Respiratory Virus Screening & Identification Kit is intended for the qualitative detection and identification of influenza A, influenza B, respiratory syncytial virus, metapneumovirus, adenovirus, and parainfluenza virus types 1, 2, and 3 in respiratory specimens, by either direct detection or cell culture method, by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs).

It is recommended that specimens found to be negative after examination of the direct specimen result be confirmed by cell culture. Negative results do not preclude respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

- Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL3+ facility is available to receive and culture human material.

II. SUMMARY AND EXPLANATION OF THE TEST

With the addition of new antiviral drugs for the treatment of influenza, more rapid and sensitive tests for respiratory virus detection and identification of the infecting viral agent has grown substantially in importance. Viral identification is becoming increasingly important in ruling out bacteria as the cause of respiratory infections. There are three influenza types: A, B, and C. Type A has counterparts in birds, horses, sea mammals and pigs as well as in humans, while types B and C are primarily known in humans.

With the potential for an additional influenza A pandemic such as occurred in 1918 when 25-35 million people died worldwide, the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of emerging influenza strains and make recommendations for suitable strains for vaccine production.

Influenza infects an estimated 120 million people in the US, Europe and Japan each year and it is estimated that there are 75,000 deaths annually in the US from pneumonia caused by influenza. Primary viral pneumonia or pneumonia from secondary bacterial infections are the primary causes of morbidity associated with influenza infection. Complications tend to occur in the young, the elderly and persons with chronic cardio-pulmonary diseases.

Pandemics of influenza A occur about every 10- to 30-years while annual epidemics are usually of either influenza A or B; however, both types may circulate concurrently. Infections are seasonal, typically extending from November to April in the northern hemisphere. Disease incubation is 1- to 3-days with rapid transmission through aerosolized droplets and fomites. The disease is characterized by sudden onset, fever, myalgia, headache and pharyngitis.

Influenza A and B are most commonly isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix™), A549/MDCK mixtures (R-Mix Too™), Rhesus MK, MDCK, MRC-5 and A549 cells.

Respiratory Syncytial Virus (RSV)

RSV (family Paramyxoviridae) is an enveloped virus with a single, negative strand RNA genome. RSV infections cause viral bronchiolitis and pneumonia in infants and the common cold in adults. RSV is the primary viral cause of lower respiratory disease in infants and young children with peak mortality due to RSV in infants and young children with peak mortality due to RSV in 3-4 month old infants. RSV is usually a seasonal (winter and early spring) infection with epidemics lasting up to 5-months. There are two major subtypes, A and B. Subtype B is characterized as the asymptomatic strain that the majority of the population experiences. More severe clinical illness involves subtype A strains which tend to predominate in most outbreaks. Re-infections do occur but tend to be limited to minor upper respiratory infections.

RSV is also recognized as a significant problem in certain adult populations including the elderly, individuals with cardiopulmonary diseases, and immunocompromised hosts.

RSV is commonly detected directly in cells from the nasopharyngeal epithelium by staining with immunofluorescent reagents although it can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), HEP2, vero, LLC-MK2 and MRC-5 cells.

Metapneumovirus

Human metapneumovirus is a respiratory viral pathogen that causes a spectrum of illnesses ranging from asymptomatic infection to severe bronchiolitis. Human metapneumovirus was first described in 2001 by researchers at the Erasmus Medical Center at Erasmus University in Rotterdam, The Netherlands. This newly recognized human viral pathogen was isolated from respiratory samples submitted for viral culture during the winter season. Half of the initial 28 hMPV isolates were cultured from patients younger than 1-year, and 96% were isolated from children younger than 6-years. Seroprevalence studies revealed that all of children aged 6- to 12-months who were tested in the 2001 study, 25% had detectable antibodies to hMPV; by age 5-years, 100% of patients showed evidence of past infection. A separate report from Australia describing three additional cases of hMPV infection supports the contention that this newly discovered virus is ubiquitous and additional information relating to pathogenesis and epidemiology continues to become available.

Metapneumovirus can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), HEP2, and A549 cells.

Adenovirus

Adenoviruses (family Adenoviridae) are non-enveloped, double stranded DNA viruses. At the present time there are 51 serotypes, further divided into 6 groups, A to F. Most adenoviruses are associated with respiratory and ocular infections. Generally, adenovirus infections in adults have a low morbidity with the exceptions of immunocompromised individuals and those living in overcrowded conditions, in which infections can cause atypical pneumonia. Virus spread is commonly through aerosolized droplets and fomites with infection of mucous membranes of the eye, respiratory tract and gut.
Adenoviruses can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), HEp2, HEK, A549 and MRC-5 cells.7

Parainfluenza Virus
Parainfluenza viruses (family Paramyxoviridae) are enveloped viruses with a single, negative strand RNA genome. The four different types cause cough and croup, or bronchiolitis, especially under two years of age, and respiratory illness in adults. Parainfluenza is the second leading cause of lower respiratory illness in children after RSV. Outbreaks caused by parainfluenza viruses usually occur in the fall during alternate years (P1 and P2) or throughout the year, with increased activity in the spring (P3).5,6

Parainfluenza viruses can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), Rhpesus MK, MRC-5 and LLC-MK2 cells. Trypsin is helpful in the medium for recovery of types 1 and 2 but not type 3.

III. PRINCIPLE OF THE PROCEDURE
The Diagnostic Hybrids, Inc. D³ Ultra 8 DFA (direct fluorescent antibody) Respiratory Virus Screening & Identification Kit uses viral antigen-specific murine monoclonal antibodies that are directly labeled with fluorescein for the rapid detection and identification of respiratory viruses. The kit includes a DFA Screening Reagent that contains a blend of murine MAb directed against eight respiratory viruses (influenza A virus, influenza B virus, RSV, MPV, adenovirus, parainfluenza virus types 1, 2, and 3) plus eight separate DFA Reagents, each consisting of MAb blends directed against a single respiratory virus. The kit can be used for direct specimen or cell culture screening and final virus identification.

The cells to be tested, either derived from a clinical specimen or cell culture, are fixed in acetone. The DFA Screening Reagent is added to the cells to determine the presence of viral antigens. After incubating at 35°C to 37°C, the stained cells are rinsed with the diluted Wash Solution (1X). A drop of the supplied Mounting Fluid is added and a coverslip is placed on the prepared cells. The cells are examined using a fluorescence microscope. Virus-infected cells will be stained with viral specific apple-green fluorescence when stained with the DFA Screening Reagent while non-infected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain. If the specimen contains fluorescent cells, the particular virus is identified using the separate DFA Reagents on new, separate cell preparations.

If on examination of a direct stained specimen, no fluorescent-stained cells are found and all the cells stain red from the Evans Blue, it is recommended that the specimen be cultured and stained using the DFA Screening Reagent. If fluorescent cells are seen, the identification of the virus is determined as described above. Cell preparations are fixed in acetone. The individual DFA Reagents are added to the cell preparations. After incubating at 35°C to 37°C, the stained cells are rinsed with the diluted Wash Solution (1X). A drop of the supplied Mounting Fluid is added and a coverslip is placed on the stained cells. The cells are examined using a fluorescence microscope for the presence of viral specific apple-green fluorescence. The unknown respiratory virus is then identified and reported.

IV. REAGENTS
A. KIT COMPONENTS

1. D³ Ultra 8 DFA Respiratory Virus Screening Reagent, 10-mL. One dropper bottle containing a blend of fluorescein-labeled murine monoclonal antibodies directed against respiratory viral antigens of influenza A virus, influenza B virus, parainfluenza virus type 1, 2, and 3. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

2. Influenza A DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by influenza virus strain Texas 1/77 (H3N2) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

3. Influenza B DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by influenza virus strain Hong Kong 5/72 (H1N1) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

4. RSV DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by respiratory syncytial virus (L.A. strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

5. Metapneumovirus DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by metapneumovirus-infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

6. Adenovirus DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by adenovirus (Type 3-GB strain and Type 6-tonsil 99 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

7. Parainfluenza 1 DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by parainfluenza virus type 1 (VP-1 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

8. Parainfluenza 2 DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by parainfluenza virus type 2 (Greer strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

9. Parainfluenza 3 DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by parainfluenza virus type 3 (C243 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

10. Normal Mouse Gamma Globulin DFA Reagent, 10-mL. One dropper bottle containing a mixture of fluorescein-labeled murine gamma globulin that has been shown to be non-reactive with any of the listed respiratory viruses. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

B. WARNINGS AND PRECAUTIONS
For in vitro diagnostic use.

1. No known test method can offer complete assurance that infectious agents are absent; therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens be handled in accordance with the OSHA Standard for Bloodborne Pathogens.

2. All specimens used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.

3. Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.

4. Avoid splashing and the generation of aerosols with clinical samples.

5. Use aseptic technique and sterile equipment and materials for all cell culture procedures.

6. Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.

7. Sodium azide is included in the 40X Wash Solution Concentrate at 4%, and in the other solutions in this kit at 0.1%. A MSDS for sodium azide or for Diagnostic Hybrids, Inc. (DHI) reagents containing sodium azide is available by contacting Diagnostic Hybrids Technical Services.

T: Sodium azide at very low levels causes damage to health.
RS15/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

a. Reagents containing sodium azide should be considered poisons. If products containing sodium azide are swallowed, seek medical advice immediately and show product container, label, or MSDS to medical personnel. (Refer to NICHS, National Institute for Occupational Safety and Health: CAS# 515-42-6; EC# 247-852-1; and also to GHS, The Globally Harmonized System of Classification and Labeling of Chemicals.)

b. Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas.

c. Any reagents containing sodium azide should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with a large volume of water to prevent azide build-up. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.

8. Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.

9. The DFA Reagents are supplied at working strength. Any dilution of the DFA Reagents will decrease sensitivity.

10. Reagents should be used prior to their expiration date.

11. Each Antigen Control Slide should be used only once. Do not re-use a Control Slide.

12. Microbial contamination of DFA Reagents may cause a decrease in sensitivity.

13. Store 1X Wash Solution and PBS (Phosphate Buffered Saline) in clean containers to prevent contamination.

14. Reusable glassware must be washed and thoroughly rinsed free of all detergents.

15. Do not expose DFA Reagents to bright light during staining or storage.

16. Use of other reagents than those specified with the components of this kit may lead to erroneous results.

C. PREPARATION OF 1X WASH SOLUTION

1. After storage at 2° to 8°C, some salts in the 40X Wash Solution Concentrate may have crystallized. Warm the solution to ambient temperature (20° to 25°C) to re-dissolve the crystals, then mix.

2. Add contents of the fully dissolved 25-mL 40X Wash Solution Concentrate to 975-mL of demineralized water.

3. Label the 1X Wash Solution with a sixty (60) day expiration date after reconstitution and store at ambient temperature.

D. STORAGE INSTRUCTIONS

<table>
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<th>Table 1: Reagent Storage Conditions</th>
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<td>9. Parainfluenza 3 DFA Reagent</td>
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<td>14. 40X Wash Solution Concentrate</td>
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<td>15. 1X Wash Solution</td>
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E. STABILITY

Reagents and components will retain their full potency through the expiration date shown on the label of each bottle when stored at recommended temperatures. Light exposure of the DFA Reagents should be kept to a minimum. Discard 1X Wash Solution if it becomes cloudy.

V. SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful respiratory virus detection. Specimen collection, specimen processing, and cell culture of viruses should be attempted only by personnel that have been trained in such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

For specimen collection and processing recommendations, refer to CLSI Approved Viral Culture Guidelines.

A. SPECIMEN COLLECTION

Aspirates and Washes containing secretions from the nasopharyngeal epithelium provide the best specimens for direct specimen testing since they will contain large numbers of epithelial cells.

Aspirates can be collected using a sterile, soft polyethylene #8 infant feeding tube attached to a disposable aspiration trap connected to a suction device. Washes can be collected by instilling and aspirating 1- to 2-mL of saline in the patient’s nostril while the patient is in a supine position.

Aspirates and washes should be diluted with equal volumes of transport medium contained in a centrifuge tube with several sterile glass beads. Swabs from nasal, throat and nasopharyngeal areas often do not contain sufficient numbers of columnar epithelial cells to allow for direct specimen detection of respiratory viruses.

B. SPECIMEN TRANSPORT AND STORAGE

All potentially infectious agents should be transported according to International Air Transport Association (IATA), International Civil Aviation Organization, (ICAO), Titles 42 and 49 of the US Code of Federal Regulations, or other regulatory requirements, as may be applicable.

Specimens should be transported to the laboratory at 2° to 8°C. This temperature can be attained by using cold packs, wet ice, foam refrigerant, or other coolants. The specimens should be processed and tested as soon as possible and then stored at 2° to 8°C.

Specimens should be stored at 2° to 8°C for no longer than 2 -days before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.

Freezing and thawing of specimens should be avoided since this will result in a loss of viability of viruses, leading to decreased sensitivity of the test.

VI. PROCEDURE

A. MATERIALS PROVIDED

1. Respiratory Virus DFA Screening Reagent
2. Influenza A DFA Reagent
3. Influenza B DFA Reagent
4. RSV DFA Reagent
5. Metapneumovirus DFA Reagent
6. Adenovirus DFA Reagent
7. Parainfluenza 1 DFA Reagent
8. Parainfluenza 2 DFA Reagent
9. Parainfluenza 3 DFA Reagent
10. Normal Mouse Gamma Globulin DFA Reagent
11. Respiratory Virus Antigen Control Slides
12. hMPV Antigen Control Slides
13. Mounting Fluid
14. 40X Wash Solution Concentrate

B. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.
2. Cell culture for respiratory virus isolation. Suggested cell lines include LLC-MK2, HEp-2, A549 cells, R-Mix and R-Mix Too MixedCells™, and primary Rhesus monkey kidney cells. All are available from DHI.
3. Live control viruses for positive culture controls: Known strains of the 8 respiratory viruses for use in monitoring the cell culture and staining procedures. Such control virus strains can be obtained from DHI.
4. Coverslips (22 x 50mm) for Antigen Control Slides and for specimen slides.
5. Universal Transport Medium. Available from DHI.
6. R-Mix Refeed Medium (for use with R-Mix and R-Mix Too MixedCells) or other standard refeed medium. Available from DHI.
7. Reagent-grade acetone (99% pure) chilled at 2° to 8°C for fixation of direct specimen slides, shell-vials and cultured cell preparations. NOTE 1: Keep the reagent-grade acetone container tightly sealed to avoid hygroscopic absorption of water, which may cause a hazy, non-specific background fluorescence.
8. Sterile graduated pipettes: 10-mL, 5-mL, and 1-mL.
9. Sterile Pasteur pipettes or other transfer pipettes.
10. Caution: One should not use solvents such as acetone with polyethylene transfer pipettes.

12. Sterile 0.45-µm syringe filter.
13. Sterile 3-mL syringe.
14. 200-mL wash bottle.
15. Bent-tip teasing needle (for removal of coverslip from a shell-vial for the typing portion of the procedure); fashion the teasing needle by bending the tip of a syringe needle or similar object (i.e., mycology teasing needle) against a bent chopstick or with a pair of forceps taking care to avoid injury.
16. Sodium hypochlorite solution (1:10 final dilution of household bleach).
17. Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom).
18. Glass microscope slides.
19. Acetone-cleaned multi-well glass microscope slides (2-well and 8-well masked slides).
20. Blocks for multi-well glass microscope slides: Two- and 8-well absorbent blotters, used to blot excess liquid from the mask to prevent spread of
21. Sterile, nylon flocked swabs or polyester swabs, which are non-inhibitory
22. Incubator, 35° to 37°C (5% CO2 or non-CO2, depending on the cell culture
25. PBS, sterile, for use in rinsing and suspending cells.
26. Aspirator Set-up: Vacuum aspirator with disinfectant trap containing
27. Wash Container: Beaker, wash bottle or Coplin jar for washing slides.
28. Fixing Container: Coplin jar, slide dish or polyethylene holder for slides
29. Inverted Light Microscope: Used for examining monolayers of cells prior
30. Light source is focused by a number of lenses and mirror(s). For
31. A low grade, yellow-green fluorescence may sometimes be seen,
32. When staining with fluorescent reagents and examining cells
33. and immediately return to refrigerator after use for storage at 2° to 8°C.
34. Add 5 -mL of PBS and vortex vigorously for 10 -to 15 -seconds.
35. Mix the suspension by pipetting up and down to re-suspend the cell
36. Add 0.5- to 1-mL of PBS.
37. Rinse the stained cells using the 1X Wash Solution. For only a few
38. rinse the supernatant for viral isolation. (Step VI.D.10 below.)
39. and collect and set aside the supernatant for viral isolation. (Step VI.D.10
40. Place the closed, humidified chamber for holding slides during staining
41. b) Bleaching or fading of the fluorescence of stained cells may occur on
42. Protect stained slides and monolayers from light as much as possible
43. Direct Specimen Testing 
44. E. DIRECT SPECIMEN TESTING
45. 1. Spot 25 µL of the prepared cell suspension on each well of a 2-well and an 8-well slide. Repeat this step for each specimen.
46. 2. Air dry the wells completely.
47. 3. Fix the cells to the slides using fresh, chilled 100% acetone for 5- to 10 -minutes at 20° to 25 °C.
48. 4. Remove the slides from the fixative and allow to air dry.
49. 5. Rinse the stained cells using the 1X Wash Solution. For only a few
50. 6. Rinse the stained cells using the 1X Wash Solution. For only a few
51. 7. Add 5 -mL of PBS and vortex vigorously for 10 -to 15 -seconds.
52. 8. Place the slides in a covered humidified chamber at 35º to 37ºC for 15-
53. 9. Rinse the stained cells using the 1X Wash Solution. For only a few
54. 10. Place the slides in a covered humidified chamber at 35º to 37ºC for 15-
55. 11. Rinse the stained cells using the 1X Wash Solution. For only a few
56. 12. Gently blot the excess demineralized water.
57. 13. Add a small drop of Mounting Fluid to each cell-containing well and
58. 14. Examine the stained, mounted cells using a fluorescence microscope
59. 15. Refer to Section VII. 'Interpretation of Results'.
60. 16. If the result is positive for respiratory virus, the staining procedure may be
61. 12. Three aspects of the fluorescence microscope that must be functioning
62. 11. Examine the positive and negative controls before examining the test
63. 10. Collect and set aside the supernatant for viral isolation. (Step VI.D.10
64. 9. Do not allow the monolayers to dry before fixing; this can lead to high
65. 8. Fixing Container: Coplin jar, slide dish or polyethylene holder for slides
66. 7. Repeat steps 4 through 6 until the mucus layer has been completely
67. 6. Place the slides in a covered humidified chamber at 35º to 37ºC for 15-
68. 5. Centrifuge at 400 to 600x g for 5- to 10 -minutes.
69. 4. Add 5 -mL of PBS and vortex vigorously for 10 -to 15 -seconds.
70. 3. Centrifuge at 400 to 600x for 5- to 10 -minutes.
71. 2. Air dry the wells completely.
72. 1. Vortex the specimen vigorously for 10- to 15 -seconds.
73. 14. Protect stained slides and monolayers from light as much as possible
during testing.
74. 13. Intense fluorescence around the periphery of slide wells is indicative
of drying of the DFA Reagent during incubation, suggesting that it
was incubated too long or the humidity was not well controlled.
75. 12. Gently blot the excess demineralized water.
76. 11. Rinse the stained cells using demineralized water. For only a few slides,
and immediately return to refrigerator after use for storage at 2° to 8°C.
77. 10. Place the slides in a covered humidified chamber at 35º to 37ºC for 15-
78. 9. Rinse the stained cells using demineralized water. For only a few
slides, a slide carrier that holds 10 to 20 slides can be placed in its
container containing 1X Wash Solution. For effective rinsing, dip the slide(s) up and down a minimum of four times.
79. 8. Place the slides in a covered humidified chamber at 35º to 37ºC for 15-
70. 7. Add 1-drop of the Normal Use Gamma Globulin DFA Reagent to
81. 5. Collect and set aside the supernatant for viral isolation. (Step VI.D.10
82. 4. Add 5 -mL of PBS and vortex vigorously for 10 -to 15 -seconds.
83. 3. Collect and set aside the supernatant for viral isolation. (Step VI.D.10
84. 2. Add 5 -mL of PBS and vortex vigorously for 10 -to 15 -seconds.
85. 1. Spot 25 µL of the prepared cell suspension on each well of a 2-well and an 8-well slide. Repeat this step for each specimen.
86. 1. Spot 25 µL of the prepared cell suspension on each well of a 2-well and an 8-well slide. Repeat this step for each specimen.
87. 1. Vortex the specimen vigorously for 10- to 15 -seconds.
88. 1. Vortex the specimen vigorously for 10- to 15 -seconds.
89. 1. Vortex the specimen vigorously for 10- to 15 -seconds.
1. Calculate the number of shell-vials needed based on the staining protocol to be used (this staining protocol requires 3 shell-vials):

- a) One additional shell-vial is required for preparing 8-well slides used to identify the viruses from positive screens.

2. Examine the monolayers for proper morphology prior to inoculation.

3. Aspirate maintenance medium from the monolayers and add 1-mL of appropriate refeed medium to each shell-vial.

4. Add 0.2 - 0.4-mL of prepared specimen to each shell-vial.

5. Centrifuge the shell-vials at 700g for 1-hour at 20°C to 25°C.

6. Place stopped shell-vials in an incubator at 35°C to 37°C.

7. When a monolayer is ready to be stained using the DFA Screening Reagent, remove the medium by aspiration and add 1-mL of PBS.

8. Swirl to mix and then aspirate.

9. Repeat the wash with another 1-mL of PBS and then aspirate.

10. Add 1-mL of fresh, chilled 100% acetone and allow to stand for 5- to 10-minutes at 20°C to 25°C.

**Caution:** Acetone is volatile and flammable; keep away from open flames.

11. Remove the fixative by aspiration.

12. Add 0.5-mL of PBS to wet the monolayer.

13. Swirl and then aspirate completely.

14. Add 4 - drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples, and rock to ensure complete coverage of the monolayer by the Reagent.

15. Place stopped shell-vials in a 35°C to 37°C incubator for 15- to 30-minutes.

16. Aspirate the DFA Screening Reagent from the monolayers.

17. Add 1-mL of the 1X Wash Solution.

18. Remove the 1X Wash Solution by aspiration, repeat the wash step and again remove by aspiration.

19. Add 1-mL of demineralized water.

20. Remove the demineralized water by aspiration.

21. Rinse the specimen slide using a 2-well and an 8-well slide. Repeat this step for each specimen.

22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (Section VI. C. 11-14, ‘Immunofluorescence Microscopy’.)

23. Refer to Section VII ‘Interpretation of Results’.

24. If the result is positive for respiratory virus, process a reserved replicate culture as a cell suspension and spot onto an 8-well specimen slide in order to identify which respiratory virus may be present (refer to Section VI.F. steps 6 through 11, for procedure to prepare a specimen slide), then:

- a) Add 1-drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Add the Metapneumovirus DFA Reagent to the well labeled ‘Neg’.

- b) For the Respiratory Virus Antigen Control Slide, add 1-drop of each individual virus DFA Reagent to its corresponding labeled well.

**NOTE:** An Antigen Control Slide should be stained only once, do not re-stain.

- c) For the hMPV Antigen Control Slide add 1-drop of the Metapneumovirus DFA Reagent to each well.

**NOTE:** An Antigen Control Slide should be stained only once, do not re-stain.

- d) Continue with steps 14 through 22 above.

**H. CELL CULTURE TESTING – Multi-well Plate**

1. Calculate the number of wells needed for the staining protocol to be used (this staining protocol requires 3-wells):

- a) One well is required for each day the culture will be screened with the DFA Screening Reagent (i.e., staining at 16- to 24-hours, and again at 48- to 72-hours, requires 2-wells).

- b) One additional well is required for preparing 8-well slides used to identify the viruses from positive screens.

- c) It is recommended that each replicate well be on a different multi-well plate. This allows each plate to be processed on the appropriate day.

2. Examine the monolayers for proper morphology prior to inoculation.

3. Aspirate maintenance medium from the monolayers and add 1-mL of appropriate refeed medium to each 24-well multi-well plate monolayer; add 0.8-mL to each 48-well plate monolayer.

4. Add 0.2 - 0.4-mL of prepared specimen to the appropriate wells of a multi-well plate.

5. Centrifuge the multi-well plates at 700g for 1-hour at 20°C to 25°C.

6. Place the covered multi-well plates in a 35°C to 37°C incubator with a humidified, 5% CO₂ atmosphere.

7. When a monolayer is ready to be stained using the DFA Screening Reagent, remove the medium by aspiration and add 1-mL of PBS.

8. Swirl to mix and then aspirate.

9. Repeat this wash with another 1-mL of PBS and then aspirate.

10. Add 1-mL of 80% aqueous acetone and let stand 5- to 10-minutes at 20°C to 25°C.

**NOTE:** Do not allow the 80% acetone fixative to remain in the polystyrene wells longer than 10-minutes since it may craze and cloud the plastic, making it difficult to examine the monolayers.

**Caution:** Acetone is volatile and flammable; keep away from open flames.

11. Remove the fixative by aspiration.

12. Add 0.5-mL of the PBS to wet the monolayer.

13. Swirl and then aspirate completely.
14. Add 4-drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples in each well of a 24-well plate; add 3-drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples in each well of a 48-well plate. Rock to ensure complete coverage of the monolayer by the Reagent.
15. Place the covered multi-well plate in a 35° to 37°C, humidified incubator for 15 to 30-minutes.
16. Aspirate the DFA Screening Reagent from the monolayers.
17. Add 1-mL of the 1X Wash Solution and mix to wash.
18. Remove the 1X Wash Solution by aspiration, repeat the wash step, and again remove by aspiration.
19. Add 1-mL of demineralized water.
20. Remove the demineralized water by aspiration.
21. Add 2- to 3-drops of Mounting Fluid to each monolayer, then cover the plate.
22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (Section VI.C. 11-14, ‘Immunofluorescence Microscopy’).
23. Refer to Section VII. ‘Interpretation of Results’.
24. If the result is positive for respiratory virus, process a reserved replicate

B. ARTIFACTS OF STAINING

I. QUALITY CONTROL
1. Reagents
a) A fresh Respiratory Virus and hMPV Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.

VII. INTERPRETATION OF RESULTS
A. EXAMINATION OF SAMPLES AND CONTROLS
1. Examine controls first to ensure proper test performance before examining patient specimens.
2. A positive reaction is one in which bright apple-green fluorescence is observed in the infected cells.
3. Non-infected cells will fluoresce dull red due to the Evans Blue counter-stain included in the DFA Reagent.
4. Examine the entire cell spot or monolayer of cells before reporting final results.
5. Do not report results for patient samples unless controls perform as expected.

B. ARTIFACTS OF STAINING
1. Dried edges of the monolayer or cell clumps may non-specifically fluoresce due to antibody trapping.
2. Dead, rounded cells may non-specifically fluoresce dull olive-green due to specimen toxicity or improper cell storage.
3. Properly controlled humidity during staining and adequate washing between steps helps prevent non-specific staining.

C. FLUORESCENT STAINING PATTERN OF RESPIRATORY VIRUS INFECTED CELLS
The following describes typical fluorescent patterns and should be used as a guide to identify specific viruses. Note that specific viral identification requires the demonstration of characteristic staining with MAbs.

The “typical” apple-green fluorescence staining pattern for each virus is as follows:

Influenza A and B Virus: The fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.

Respiratory Syncytial Virus: The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.

Adenovirus: The fluorescence is cytoplasmic and punctate or bright nuclear or both.

Parainfluenza virus types 1, 2, and 3: The fluorescence is cytoplasmic and punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia.

Metapneumovirus: The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.

Co-infection with more than one infecting virus present in the specimen has been reported in a number of studies. The presence of multiple viruses is indicated when more than one well of the 8-well slide has fluorescent cells. The identification of the viruses is based on the individual virus DFA Reagents showing fluorescence. In such a case, it should be reported as “… and … detected by direct specimen testing” or “… and … isolated by cell culture.”

D. RESULTS FROM DIRECT SPECIMEN STAINING
1. Evaluation of sample suitability
a) Each stained patient specimen should be reviewed for the presence of columnar epithelial cells (cells that are taller than they are wide). The quality of the specimen with respect to the number of epithelial cells in the sample can be assessed by examining different fields at a magnification of 200X.

b) A satisfactory specimen should have at least 2 columnar epithelial cells per field. A negative result is indicated by the absence of fluorescent cells in a minimum sampling of 20 columnar epithelial cells.

c) An inadequate specimen is indicated by fewer than 20 columnar epithelial cells present in the sample, in which case the test is considered invalid. A new specimen should be obtained and tested or cell culture for virus isolation should be initiated from the remaining specimen.

2. Reporting Results of Direct Specimen Staining
a) The entire cell spot must be examined for virus-infected, apple-green fluorescent cells.

b) A satisfactory specimen with no fluorescent cells observed should be reported as “No influenza A, influenza B, respiratory syncytial virus, metapneumovirus, adenovirus, parainfluenza type 1, parainfluenza type 2, or parainfluenza type 3 viral antigens detected by direct specimen testing”. However, such negative results should be confirmed using cell culture.

c) Specimens negative by direct specimen testing but yielding positive culture results should be reported as “… isolated by cell culture”, where “…” is the appropriate virus, e.g., influenza A, influenza B, metapneumovirus, adenovirus, respiratory syncytial virus, parainfluenza type 1, 2, or 3 (Section VII.E. ‘Results from Culture Isolation / Confirmation’, below).

d) If apple-green fluorescent cells are found, the identification of the virus(es) may be based on the individual DFA Reagents (according to Section VI.E.). The individual virus DFA Reagent that yields fluorescent cells represents the identification of the respiratory virus. In such a case, it should be reported as “… detected by direct specimen testing”, where “…” is the appropriate virus, e.g., influenza A, influenza B, metapneumovirus, adenovirus, respiratory syncytial virus, parainfluenza virus type 1, 2, or 3.

E. RESULTS FROM CULTURE ISOLATION/CONFIRMATION
1. The entire cell spot or monolayer of cells must be examined for virus-infected, apple-green fluorescent cells. If no fluorescent cells are found, the results should be reported as, “No influenza A, influenza B, respiratory syncytial virus, metapneumovirus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, or parainfluenza virus type 3 isolated in cell culture.”

2. If apple-green fluorescing cells are found, the identification of the virus(es) may be based on the individual DFA Reagents (according to the appropriate Sections VI. F., G., and H.). In such cases, identification of the viral antigen(s) should be reported as “… isolated in cell culture”, where “…” is the appropriate virus, e.g., influenza A virus, influenza B virus, respiratory syncytial virus, metapneumovirus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, or parainfluenza virus type 3.

VIII. LIMITATIONS OF PROCEDURE
1. Inappropriate specimen collection, storage, and transport may lead to false negative culture results.

2. Assay performance characteristics have not been established for direct specimen staining on specimens other than respiratory specimens. It is
the user’s responsibility to establish assay performance for specimens other than respiratory specimens.
3. Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
4. Detection of viruses will vary greatly depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of virus infection. Results of the test should be interpreted in conjunction with information available from epidemiological studies, clinical evaluation of the patient and other diagnostic procedures.
5. The effects of antiviral therapy on the performance of this kit have not been established.
6. The MAbs used in this kit are from hybridomas created using viral infected cells as the immunogen. The specific viral antigens detected by the antibodies are undetermined.
7. Since the MAbs have been prepared using defined virus strains, they may not detect all antigenic variants or new strains of the viruses, should they arise. MAbs may fail to detect strains of viruses which have undergone minor amino acid changes in the target epitope region.
8. The MAbs used in this kit are not group specific and therefore cannot be used to differentiate among the different types of adenovirus and RSV.
9. The viral antigens detected in some direct specimens may be from non-viable virus and cannot be isolated by culture. This is particularly true of RSV which is known for its instability and loss of viability.
10. A negative direct specimen should be inoculated into an appropriate cell culture and incubated to isolate and identify any respiratory virus that may be present in the specimen.
11. A negative result on a direct or cultured specimen does not rule out the presence of virus.
12. Performance of the kit can only be assured when components supplied in the assay are those supplied by DHI.
13. Prolonged storage of the DFA Reagents under bright light will decrease the staining intensity.
14. Light background staining may occur with specimens contaminated with Staphylococcus aureus strains containing large amounts of protein A. Protein A will bind to the Fc portions of conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., S. aureus-bound fluorescence appears as small (~1 micron diameter), bright dots. Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution.

IX. EXPECTED VALUES

Respiratory virus infections are often seasonal, with influenza typically extending from November to April in the northern hemisphere, and adenovirus infections occurring more often during late winter to early summer. RSV usually occurs during the winter to early spring months of 2007/2008. Prevalence of the respiratory viruses within the population of specimens that was prospectively collected and the cells directly tested is noted in Table 3 below.

The clinical studies described in Section X (‘Specific Performance Characteristics’) were comprised of respiratory specimens collected during the winter to early spring months of 2007/2008. Prevalence of the respiratory viruses within the population of specimens that was prospectively collected and the cells directly tested is noted in Table 2 below.

| TABLE 2: Prevalence of the Respiratory Viruses within the Study Population |
|-----------------|-------------|-------------|-------------|-------------|-------------|------------------|-------------|
| Expected Values | Adeno       | Flu A       | Flu B       | Para 1      | Para 2      | Para 3         | RSV          | MPV          |
| Direct Specimens |             |             |             |             |             |                 |             |             |
| (n = 516)       | 23          | 71          | 5           | 4           | 1           | 4               | 74           | 12           |
| Prevalence      | 4.5%        | 13.8%       | 1.0%        | 0.8%        | 0.2%        | 0.8%            | 14.3%        | 2.3%         |

X. SPECIFIC PERFORMANCE CHARACTERISTICS

A. CLINICAL PERFORMANCE CHARACTERISTICS

1. Study Site 1

The study consisted of a total of 300 fresh specimens submitted, January through February 2007, to the laboratory for respiratory virus testing. Slides were prepared from PBS-washed cells from the fresh specimens and fixed in accordance with the procedure in the Comparator product insert (same procedure for both Subject and Comparator devices).

The slides were stored at -70°C until testing was performed. The preparation of specimen slides, fixing in acetone, and freezing is common laboratory practice in order to batch test at a later time. With the fixation in acetone, prior to freezing, the cellular morphology is maintained during freezing and on thawing.

Table 3 shows the age distribution for individuals studied at Study Site 1.

<table>
<thead>
<tr>
<th>TABLE 3: Study Site 1 - Age Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group</td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>≤1 month</td>
</tr>
<tr>
<td>&gt;1 month - 2 years</td>
</tr>
<tr>
<td>&gt;2 - 12 years</td>
</tr>
<tr>
<td>&gt;12 - 21 years</td>
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<tr>
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<td>31 - 40 years</td>
</tr>
<tr>
<td>41 - 50 years</td>
</tr>
<tr>
<td>51 - 60 years</td>
</tr>
</tbody>
</table>

The following tables detail the results from Study Site 1:

Table 4 compares the results of the D³ Ultra 8 Screening Reagent (D³ Ultra 8 DFA Respiratory Virus Screening Reagent) with those of the D³ Ultra Screening Reagent (D³ Ultra DFA Respiratory Virus Screening Reagent) for detecting the seven respiratory viruses identified by the D³ Ultra Screening Reagent in cells derived from a specimen.

Table 5 compares the D³ Ultra 8 Screening Reagent with those of the D³ Metapneumovirus DFA Reagent for detecting metapneumovirus in cells derived from a specimen.

<table>
<thead>
<tr>
<th>TABLE 4: Study Site 1 – Comparison of D³ Ultra 8 and the D³ Ultra for Detecting all Seven Respiratory Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Specimen</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>D³ Ultra 8 Screening Reagent</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Positive Percent Agreement (PPA)</td>
</tr>
<tr>
<td>95% CI-PPA</td>
</tr>
<tr>
<td>Negative Percent Agreement (NPA)</td>
</tr>
<tr>
<td>95% CI-NPA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 5: Study Site 1 – D³ Ultra 8 Identification of Metapneumovirus Positive Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Specimen</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>D³ Ultra 8 Screening Reagent</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Positive Percent Agreement (PPA)</td>
</tr>
<tr>
<td>95% CI-PPA</td>
</tr>
<tr>
<td>Negative Percent Agreement (NPA)</td>
</tr>
<tr>
<td>95% CI-NPA</td>
</tr>
</tbody>
</table>

Study Site 1 - CONCLUSION

A variety of viral respiratory pathogens were detected: respiratory syncytial virus [prevalence 2.6% (9/300)], influenza A virus [prevalence 5% (15/300)], influenza B virus [prevalence 0.5% (1/300)], metapneumovirus [prevalence 1% (5/300)], adenovirus [prevalence 2% (6/300)], parainfluenza type 1 virus [prevalence 2% (6/300)] and parainfluenza type 3 virus [prevalence 3% (10/300)]. No co-infections were detected.

The D³ Ultra 8's ability to identify the seven viruses in direct specimens was compared to the D³ Ultra's ability. The positive percent agreement was 100% (95% CI range of 98.1% to 100%). The negative percent agreement was 100% (95% CI range of 98.1% to 100%). The D³ Ultra 8's ability to identify metapneumovirus in direct specimens was compared to the D³ Metapneumovirus DFA Reagent's ability. The positive percent agreement was 100% (95% CI range of 98.8% to 100%). The negative percent agreement was 100% (95% CI range of 97.9% to 100%).

For Study Site 1, the performance results of the D³ Ultra 8, when compared to those of the Comparator devices, D³ Ultra and D³ Metapneumovirus DFA Reagent, demonstrate that the devices detect respiratory virus antigens in similar manners, and that the addition of the metapneumovirus MAbs do not adversely impact the other MAbs for the detection of the other respiratory viruses found (i.e., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus and parainfluenza virus types 1 and 3). No increase in background was seen with the D³ Ultra 8.
2. Study Site 2

Two hundred and sixty-eight (268) specimens were processed for direct specimen testing in accordance with the procedure in the D³ Ultra product insert (same procedure for both Subject and Comparator devices). The first forty-eight specimens were not included in analysis due to a processing issue; the cells were not rinsed sufficiently and did not remain fixed on the slide. An additional rinse step was added to the procedure for the remaining 220 specimens; the cells remained fixed to the slides. Of these 220 specimens, 4 had insufficient cells (<20) present to be interpreted. A total of 216 specimens remained for final analysis.

Two hundred and sixty-eight (268) specimens were processed for cell culture testing in accordance with the procedure in the D³ Ultra product insert (same procedure for both Subject and Comparator devices). The specimens were processed for cell culture using R-Mix Too™ FreshCells™ in 48/24-well multi-well plates. Thirteen (13) specimens were toxic and 1 was contaminated in cell culture. A total of 252 specimens remained for final analysis.

Table 6 shows the age distribution for individuals studied at Study Site 2:

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number</th>
</tr>
</thead>
<tbody>
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<td>0 - 1 month</td>
<td>2</td>
</tr>
<tr>
<td>&gt;1 month - 2 years</td>
<td>78</td>
</tr>
<tr>
<td>&gt;2 - 12 years</td>
<td>38</td>
</tr>
<tr>
<td>&gt;12 - 21 years</td>
<td>35</td>
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<tr>
<td>22 - 30 years</td>
<td>39</td>
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<tr>
<td>31 - 40 years</td>
<td>19</td>
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<tr>
<td>41 - 50 years</td>
<td>19</td>
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<tr>
<td>51 - 60 years</td>
<td>11</td>
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<tr>
<td>61 - 70 years</td>
<td>12</td>
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<tr>
<td>71 - 80 years</td>
<td>7</td>
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<td>81 - 90 years</td>
<td>5</td>
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<tr>
<td>&gt;90 years</td>
<td>1</td>
</tr>
<tr>
<td>Unknown age</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>268</td>
</tr>
</tbody>
</table>

The following tables detail the direct specimen testing results for Study Site 2:

Table 7 compares the results of the D³ Ultra Screening Reagent with those of the Comparator devices, D³ Metapneumovirus DFA Reagent, demonstrate that the devices detect respiratory virus antigens in direct specimens in similar manners, and that the addition of the metapneumovirus MAbs do not adversely impact the other MAbs for the detection of the other respiratory viruses found (i.e., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus and parainfluenza virus types 1 and 3). No increase in background was seen with the D³ Ultra.

The following tables detail the cell culture testing results for Study Site 2:

Table 8 shows the comparison of the D³ Metapneumovirus DFA Reagent for detecting metapneumovirus in cells derived from a culture.

Table 9 compares the results of the D³ Ultra 8 Screening Reagent with those of the D³ Ultra 8 REAGENT for detecting the seven respiratory viruses identified by the D³ Ultra 8 Screening Reagent in cells derived from culture.

Table 10 compares the D³ Ultra 8 Screening Reagent with those of the D³ Metapneumovirus DFA Reagent for detecting metapneumovirus in cells derived from a culture.

Study Site 2 Culture Confirmation - CONCLUSION

A variety of viral respiratory pathogens were isolated: respiratory syncytial virus [prevalence 13.4% (23/172)], influenza A virus [prevalence 20.4% (44/216)], influenza B virus [prevalence 1.9% (4/216)], metapneumovirus [prevalence 2.8% (7/252)], adenovirus [prevalence 1.6% (4/252)], and parainfluenza type 2 virus [prevalence 0.4% (1/252)]. There were two co-infections as follows: two (2) influenza A virus + respiratory syncytial virus.

The D³ Ultra 8's ability to identify the seven viruses in cells derived from a culture was compared to the D³ Ultra's ability. The positive percent agreement was 100% (95% CI range of 96.4% to 100%). The negative percent agreement was 100% (95% CI range of 96.5% to 100%).

For Study Site 2, the performance results of the D³ Ultra 8, when compared to those of the Comparator devices, D³ Ultra and D³ Metapneumovirus DFA Reagent, demonstrate that the devices detect respiratory virus antigens in direct specimens in similar manners, and that the addition of the metapneumovirus MAbs do not adversely impact the other MAbs for the detection of the other respiratory viruses found (i.e., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus and parainfluenza virus types 1 and 3). No increase in background was seen with the D³ Ultra 8.

B. ANALYTICAL PERFORMANCE CHARACTERISTICS – CROSS-REACTIVITY TESTING

Diagnostic Hybrids, Inc. D³ Ultra 8 DFA Respiratory Virus Screening & Identification Kit DFA Reagents were tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 21 host culture cell types or for 62 virus strains (cultured and processed for staining). Twenty-five (25) bacterial strains, one yeast, three Chlamydia sp. and one protozoan were evaluated for cross-reactivity including Staphylococcus aureus, a protein-A-producing bacterium. Staining of S. aureus appeared as small points of fluorescence while all other bacterial...
cultures were negative. [Table 11, Cross-Reactivity Study Results. The table indicates which organisms were reactive with which DFA Reagent.]

Stringent conditions for cross-reactivity testing were achieved by using high concentration of the DFA Reagents and high titer of microorganisms. The DFA Reagents (i.e., directly fluoresceinated monoclonal antibodies) were prepared at 1:5X the concentration that is provided in the kit. Each of the tested viruses was prepared as infected cell monolayers (250 infected cells per 400X microscope field), then stained with the 1.5X DFA Reagents and high titers of microorganisms. The stringent conditions for cross-reactivity testing were achieved by using high concentrations of the DFA Reagents, then spotted on microscope slides (yielding > 150 bacteria per 400X microscope field), then stained with the 1.5X DFA Reagents according to the procedure detailed in this product insert. Bacterial strains were cultured, processed as suspensions, then spotted on microscope slides (yielding > 150 bacteria per 400X microscope field), then stained with the 1.5X DFA Reagents according to the procedure in this product insert. Cell cultures were stained as confluent monolayers.

### Table 11: Cross-Reactivity Study Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain or Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>+ 714 TCID₅₀</td>
</tr>
<tr>
<td>Type 2</td>
<td>+ 714 TCID₅₀</td>
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<tr>
<td>Type 3</td>
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<td>Type 41</td>
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</tr>
</tbody>
</table>

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Staining of *S. aureus* appeared as small points of fluorescence while all other cultures were negative. This will be noted in labeling in the section "Limitations of the Assay": The Protein A produced by the bacterium, *Staphylococcus aureus*, will bind the Fc portion of some of the fluorescein-labeled monoclonal antibodies used in this kit. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., *S. aureus*-bound fluorescence appears as small (~1 micron diameter), bright dots. Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution.
TABLE 11: Cross-Reactivity Study Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain or Type</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt;/Source/CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia psittaci</td>
<td>-</td>
<td>Control slide</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>-</td>
<td>Control slide</td>
</tr>
<tr>
<td>Yeast</td>
<td>Candida glabrata</td>
<td>8.7x10&lt;sup&gt;6&lt;/sup&gt; CFU</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Trichomonas vaginalis</td>
<td>-</td>
</tr>
</tbody>
</table>

* Although this test has been shown to detect the 2009 H1N1 influenza virus in two cultured isolates, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D³ Ultra 8 DFA Respiratory Virus Screening & Identification Kit can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

XI. BIBLIOGRAPHY